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2.	Patent application number *(The Patent Office will fill in this part)*	0403406.2 17 FEB 2004
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	BioTranSys Limited C/O Jackson Bly 110 Nottingham Road Chilwell Nottingham, NG9 6DQ United Kingdom
	Patents ADP number *(if you know it)*	8810566001
	If the applicant is a corporate body, give the country/state of its incorporation	England
4.	Title of the invention	Preparation of Carriers for Drug Delivery and Other Therapeutic Applications
5.	Name of your agent *(if you have one)*	AdamsonJones
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Broadway Business Centre 32a Stoney Street Nottingham NG1 1LL
	Patents ADP number *(if you know it)*	~~07979487001~~ 7975907001

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a) any applicant named in part 3 is not an inventor, or
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Patents Form 1/77

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Continuation sheets of this form	
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Claim(s)	4 DL
Abstract	0
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10. If you are also filing any of the following, state how many against each item.

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Translation of priority documents	
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11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 17 February 2004

12. Name, daytime telephone number and email address, if any, of person to contact in the United Kingdom

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Title – Preparation of Carriers for Drug Delivery and Other Therapeutic Applications

This invention relates to the field of drug delivery, and in particular to processes and compositions for the delivery of therapeutic agents either intravenously or topically. The invention describes a process for preparing protein carrier systems for the attachment or inclusion of therapeutic agents for the treatment of disease states, management of bleeding and tissue repair.

The invention relates to the formation of a range of drug delivery vehicles, from soluble small protein polymers to gels, using an easily-performed chemical procedure. The process is simple and scaleable for commercial use.

The invention relates in the first instance to the formation of a protein polymer suitable for parenteral delivery. The polymer can be used to target specific sites in the body and deliver one or more therapeutically active agents, from small drugs to large proteins. Attachment is preferably by chemical linkage, or by adsorption, or by inclusion of the active agent into the polymer during formation. More than one agent can be delivered on the same polymer.

This invention also relates to the formation of gels suitable for topical administration, eg to external wounds, burns and ulcers amongst other applications. The application can be either as an inclusion to a bandage, or as a dressing, or as a spray or solution applied directly to the skin and allowed to gel. The gels may also be used internally as vehicles for the slow or controlled release of drugs, and may also be used to prevent or inhibit tissue adhesions following surgical procedures, by forming a barrier between adjacent tissue membranes.

This invention also describes the formation of a "bioadhesive" compound suitable for the coating of surgical implements eg catheters or stents, and glass or plastic

Particularly preferred proteins are albumins.

Where the protein polymers prepared in accordance with the invention are intended for administration to the human body, the protein used is preferably of human origin, ie actually derived from humans, or is identical (or substantially so) in structure to protein of human origin. A particularly preferred protein is thus human serum albumin.

Human serum albumin may be serum-derived, for instance obtained from donated blood. Human serum albumin is readily available as a fractionated blood product and has been safely used for many years for intravenous delivery as a blood expander. However, in order to eliminate or reduce the risk of transmission of potential contaminants, eg viral or other harmful agents, that may be present in blood-derived products, as well the potential limitations on supply associated with material isolated from donated blood, the protein, eg human serum albumin, may be a recombinant product derived from microorganisms (including cell lines), transgenic plants or animals that have been transformed or transfected to express the protein.

For veterinary use, non-human animal-derived protein may be used, as appropriate. Examples of such proteins include horse serum albumin, dog serum albumin etc.

Mixtures of proteins, ie more than one different protein, may be used.

Functional groups on the protein molecules with which the cross-linking agent may react include amino groups. Preferred proteins therefore include proteins with relatively high proportions of amino acid residues that include free amino groups, particularly primary amino groups. One example of such an amino acid residue is lysine, and so particularly preferred proteins for use in the invention include proteins including lysine residues, especially proteins with high proportions of

Parameters that can be varied to achieve these differing results include the choice of protein starting material, the choice of cross-linking agent, the concentrations of the various reactants, the reaction temperature and duration of the various reaction steps.

Using the method of the invention, protein polymer gels can be produced with differing consistencies (soft to hard), strengths (which may be significant for applications of the gels as bioadhesives or sealants) and porosities (which may be important in applications where the gel is used for controlled drug release). The speed of gel formation can also be varied over a wide range, from seconds to minutes to hours, by controlling the ratio of reagents used to form the gel and the temperature.

In general, an increase in the carbon chain length of the dicarboxylic acid has been found to increase the apparent hardness of the gel and the speed at which the gel forms. It has also been found that the gelling time is increased by increasing the concentration of protein in the reaction.

In order to facilitate reaction of the cross-linking agent with the protein molecules, it will generally be desirable for the cross-linking agent to be activated, ie for the carboxyl groups of the cross-linking agent to be converted to groups of greater reactivity towards groups in the protein. Suitable activation chemistries will be familiar to those skilled in the art, and include the formation of active ester groups.

One particular class of activators is carbodiimide compounds, and a particularly preferred activator for use in the invention is ethyl[dimethylaminopropyl]-carbodiimide (EDAC). In one embodiment of the invention the dicarboxylic acid (preferably C6-C10 in length) is added to the protein solution. EDAC is added to the mixture and the reaction is allowed to proceed. The concentration of the protein solution, the proportion of dicarboxylic acid to protein, the amount of EDAC

and the time are all important to the desired result. The EDAC activates –COOH groups and allows linking with free amine groups on the protein.

The control of the reaction means that the polymerisation can be controlled to give soluble polymers, insoluble particles or gels from the same reaction mixture. Greater than 95% conversion of the starting protein concentration to a polymer may be obtained, and up to 100% conversion into a gel.

The omission of the dicarboxylic acid cross-linking agent, and the use of EDAC alone, leads only to partial polymerisation over a period of several hours to days, with a lower yield of polymer compared to that obtained when a dicarboxylic acid is used.

In general, the method according to the invention will be carried out in solution. Preferably, an activating agent, eg EDAC, is added to a solution of the protein and the dicarboxylic acid. Although in principle it is also possible to firstly activate the dicarboxylic acid with EDAC and then to add the activated cross-linking agent to the protein solution, this has been found in practice not to produce results as good as those obtained by adding EDAC to the mixture of protein and cross-linking agent.

For clinical applications, it may be desirable to formulate the reactants as a mixed dry powder to which water, saline or a buffer solution is added immediately prior to application.

The protein polymers produced in accordance with the invention have many applications.

First, the protein polymers may be used as drug delivery vehicles. The polymers may be prepared in soluble form suitable for intravenous administration.

Alternatively, the protein polymers may have the form of gels suitable for topical application.

One or more therapeutically active or bioactive components may be associated with the protein polymer, either by covalent bonding thereto, or by adsorption or entrapment therein. One such active component may be for site-specific targeting, eg a monoclonal antibody specific for a disease site such as a tumour.

Active components that are bound to, or associated with, the protein polymer may include fibrinogen, fibronectin, F-VIII, F-IX, tissue repair agents, haemostatic agents and active fragments thereof, and cytotoxic agents. Other components that may be associated with the protein polymer include radionuclides, gene vectors and vaccines.

Protein polymers according to the invention may be used to control bleeding as an intravenous platelet substitute, or as a topical agent for the management of bleeding and wound repair.

The protein polymer may also be used in diagnostic applications, or as a growth medium for cells, eg stem cells.

An important area of application of the protein polymer according to the invention is in the treatment of wounds, burns or ulcers. Protein polymer gels may be impregnated into bandages, or may be applied as gel sheets (either with or without a supporting substrate) directly to a wound site. The gels may be used to pack deep wounds or ulcers.

The protein polymer may be administered to the body in the form of a solution, gel or a powder.

Gels may also be formed by the application of the reactants separately to the wound site such that the gel is formed *in situ*. The reactants may be applied in the

form of solutions, or in powder form. Suitable powders may be prepared by spray-drying or freeze-drying. Application of solutions to a wound site may be by pouring, painting or spraying of the solutions.

The protein polymer according to the invention may also be useful as a coating for devices such as catheters and stents that are intended for implantation in the body. Such a coating may have bioadhesive properties that aid retention of the device in the desired location.

The invention will now be described in greater detail, by way of illustration only, with reference to the following non-limitative Examples.

Abbreviations

DMSO	Dimethylsulfoxide
EDAC	Ethyl[dimethylaminopropyl]carbodiimide
EMCH	N-[maleimidocaproic acid]hydrazide
HSA	Human serum albumin
PBS	Phosphate buffered saline

Example 1

Formation of soluble protein polymers

1.1 Formation of a soluble polymer of HSA using sebacic acid

Sebacic acid (146mg) in 2.5ml DMSO was added to 10ml HSA in 30 ml PBS buffer with stirring until the solution became clear.

EDAC (276mg) in 7.5ml PBS buffer was added to the solution and stirred for 16 hours (overnight). The resulting solution was centrifuged to remove the small

amount of insoluble polymer. The soluble fraction was gel-filtered on a Sepharose 6B column using standard conditions. A_{280nm} plotted v eluted volume.

1.2 Preparation of a soluble polymer of HSA using adipic acid

A solution of 26.3mg (180µmol) adipic acid in 1ml 50% ethanol was added to a stirred solution of 5ml (15µmol) HSA, and stirring continued until the solution became clear.

EDAC, 69mg (360µmol) in 4ml PBS buffer was added dropwise to the solution with stirring. The resulting solution was stirred for a further 2 hrs.

The resulting solution was centrifuged to remove the small amount of insoluble polymer. The soluble fraction was gel-filtered on a Sepharose 6B column using standard conditions. A_{280nm} plotted v eluted volume.

Example 2

Formation of an insoluble particle

Insoluble protein polymer particles can be prepared by methods analogous to those of Example 1, but with concentration of dicarboxylic acid cross-linking agent and/or increased reaction time.

Example 3

Formation of protein polymer gels

3.1 Preparation of HSA polymer gel using sebacic acid and high concentration of HSA solution

A solution of 48.5mg (240µmol) sebacic acid in 1ml DMSO was added to 4ml (12µmol) human serum albumin (HSA). The solution was stirred until

it became clear. A solution of 92mg (480µmol) of EDAC in 2ml water was added. The final concentration of HSA in the reaction was 114mg/ml.

The resulting mixture became a gel after 30s.

3.2 Preparation of HSA polymer gel using sebacic acid and low concentration of HSA solution

The same experimental procedure was used as for Example 3.1 except that the final concentration of HSA was 72mg/ml.

The resulting mixture became a gel in less than 5 minutes.

3.3 Preparation of HSA polymer gel using activator, no dicarboxylic acid cross-linking agent and high concentration of HSA solution

EDAC, 92mg (480µmol) in 2ml water, was added to 4ml (12µmol) of HSA, and the resulting solution was stirred at room temperature.

The resulting mixture became a gel after 2hours.

3.4 Preparation of HSA polymer gel using adipic acid and high concentration of HSA solution

Adipic acid, 35mg(240 µmol), was dissolved in 4ml (12µmol) HSA.
A solution of 92mg(480 µmol) EDAC in 2ml water was added as above.

The resulting mixture became a soft gel polymer after 2 minutes.

3.5 Preparation of polymer gel using sebacic acid and human haemoglobin

Human haemoglobin (100mg) was dissolved in 4ml PBS buffer and stirred for 5minutes. A solution of 7.2mg sebacic acid in 0.25ml DMSO was added, again with stirring. A solution of 13.8mg EDAC in 0.75ml PBS buffer was added dropwise and the resulting solution was stirred for 2hrs. The brown turbid solution was centrifuged to remove soluble product.

The molar ratio of hemoglobin/sebacic acid/EDAC was 1/20/40 respectively.

Example 4

Linking of fibrinogen to soluble HSA protein polymer to produce a platelet substitute

i) Preparation of protein polymer

Sebacic acid (30 mg) in 1.25 ml DMSO was added to 5ml HSA in 15 ml PBS buffer and stirred until the solution became clear. EDAC (57 mg) in 4 ml PBS buffer was added to the HSA solution and stirred at room temperature for 3 hours.

ii) Thiolation of protein polymer

2-Iminothiolane (210 mg) was added as solid to the polymer solution, and left in the dark at room temperature for 1.5 hours. The polymer was then desalted by gel filtration on a Sephadex G25 column.

iii) Activation of fibrinogen

Fibrinogen (750 mg) in 10 ml 0.05 M phosphate buffer was mixed with 2.5 ml 100mM sodium periodate in 0.1M sodium acetate buffer and stirred in the dark at room temperature for 30 minutes. The activated fibrinogen was then desalted by gel filtration on a Sephadex G25 column. EMCH (11 mg) was then added and allowed to react in the dark at room temperature for 2 hours.

iv) Conjugation of activated fibrinogen with protein polymer

The solution of activated EMCH-fibrinogen solution was added to the iminothiolated polymer solution and stirred overnight. The resulting solution was centrifuged to remove any insoluble material and then gel-filtered on a Sepharose6B column.

Example 5

The effect of cross-linker chain length on gel formation

Using methods analogous to those of Example 3, protein polymer gels were prepared using HSA at various concentrations and various dicarboxylic acid cross-linking agents.

The results are summarised in the following Table:

Dicarboxylic acid	[HSA] mg/ml	Time to form gel	Type of Gel
C6 Adipic	67	>1day	No gel
C6 Adipic	114	6min	Soft gel
C6 Adipic	133	2min	Soft gel
C8 Suberic	73	15min	Very soft gel
C8 Suberic	114	2min	Hard gel
C10 Sebacic	73	3min	Hard gel
C10 Sebacic	114	0.5min	Hard gel

Claims

1. A method of forming a protein polymer, which method comprises reacting a protein with a dicarboxylic acid cross-linking agent, or an activated derivative thereof.

2. The method as claimed in Claim 1, which is carried out in solution.

3. The method as claimed in Claim 1 or Claim 2, wherein an activating agent is added to a solution of the protein and the dicarboxylic acid cross-linking agent.

4. The method as claimed in any preceding claim, wherein the protein is selected from globular proteins and fibrous or structural proteins, and mixtures thereof.

5. The method as claimed in Claim 4, wherein the protein is a globular protein.

6. The method as claimed in Claim 5, wherein the protein is a serum protein.

7. The method as claimed in Claim 6, wherein the serum protein is albumin.

8. The method as claimed in Claim 7, wherein the protein is human serum albumin.

9. The method as claimed in Claim 8, wherein the protein is of human origin or is identical (or substantially so) in structure to protein of human origin

10. The method as claimed in Claim 9, wherein the protein is a recombinant protein.

11. The method as claimed in Claim 10, wherein the protein is recombinant human serum albumin.

12. The method as claimed in any preceding claim, wherein the dicarboxylic acid cross-linking agent has the formula

$$HOOC(CH_2)_nCOOH$$

wherein n is from 0 to 20.

13. The method as claimed in Claim 12, wherein n is from 2 to 12.

14. The method as claimed in Claim 13, wherein n is from 4 to 8.

15. The method as claimed in Claim 12, wherein the dicarboxylic acid cross-linking agent is selected from the group consisting of oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid and sebacic acid.

16. The method as claimed in any preceding claim, wherein the dicarboxylic acid cross-linking agent is activated using a carboidiimide activating agent.

17. The method as claimed in Claim 16, wherein the carbodiimide activating agent is ethyl[dimethylaminopropyl]carbodiimide.

18. The method as claimed in any preceding claim, comprising the further step of associating with the protein polymer one or more therapeutically active or bioactive components.

19. The method as claimed in Claim 18, wherein the therapeutically active or bioactive components are associated with the protein polymer by being covalently bound thereto.

20. The method as claimed in Claim 18 or Claim 19, wherein the one or more therapeutically active or bioactive components are selected from the group consisting of fibrinogen, fibronectin, F-VIII, F-IX, tissue repair agents, haemostatic agents and active fragments thereof, cytotoxic agents, radionuclides and vaccines.

21. A protein polymer prepared by any one of the preceding claims.

22. The protein polymer as claimed in Claim 21, which is in the form of a solution.

23. The protein polymer as claimed in Claim 21, which is in the form of insoluble particles.

24. The protein polymer as claimed in Claim 21, which is in the form of a gel.

25. The use of a protein polymer as claimed in any one of Claims 21 to 24 in the delivery of one or more therapeutically active or bioactive components to the body.

26. The use of a protein polymer as claimed in Claim 24 in the topical treatment of a wound, burn or ulcer.

27. The use as claimed in Claim 26, wherein the protein polymer is applied to the wound, burn or ulcer as a preformed gel.

28. The use as claimed in Claim 26, wherein a protein and a dicarboxylic acid cross-linking agent are applied in solution to the wound, burn or ulcer, and the protein polymer is formed *in situ*.

29. The use of a protein polymer as claimed in any one of Claims 21 to 24 as a coating for a device to be implanted in the body.

30. The use of a protein polymer as claimed in Claim 21 as a platelet substitute.

31. A method of treatment of the human or animal body, which method comprises the administration to the body of a protein polymer as claimed in any one of Claims 21 to 24.

32. The method as claimed in Claim 31, wherein the protein polymer is administered intravenously.

33. The method as claimed in Claim 31, wherein the protein polymer is administered topically.

34. The method as claimed in any one of Claims 31 to 33, wherein the protein polymer is administered in the form of a solution.

35. The method as claimed in Claim 33, wherein the protein polymer is administered in the form of a powder.

36. The method as claimed in Claim 33, wherein the protein polymer is administered in the form of a gel.

37. The method as claimed in Claim 33, wherein the protein and the dicarboxylic acid cross-linking agent are administered to the body, such that the protein polymer is formed *in situ*.